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NIOSH "PROJECT"

USING TOXICOLOGY TO IDENTIFY CANDIDATE GENES FOR TESTING GENE:ENVIRONMENT INTERACTIONS IN ESTIMATING HUMAN RISKS

Goals and Objectives. The goal of this project is to estimate the impact of genetic polymorphisms on estimates of human health risks associated with exposures to industrial solvents. Mechanistic toxicology is a valuable source of information to generate testable hypotheses of the impact of genetic polymorphisms on population responses to toxic chemical exposures. We propose to utilize data from ongoing studies in our group to test the nature and magnitude of interactions between lead exposures and polymorphisms in endothelial nitric oxide synthase (eNOS). The objectives are as follows:

1. estimate the prevalence in the US population of Glu298Asp polymorphisms in eNOS, an enzyme responsible for the synthesis of nitric oxide by endothelial cells; NO plays a major role in regulating blood pressure through its effects on vascular smooth muscle.
2. Test the effects of lead in vitro on variant eNOS proteins associated with these polymorphisms by in vitro studies of guinea pig and human endothelial cells; in guinea pigs, test the effects of lead in vivo on eNOS activity and on blood pressure.
3. predict the risk of adverse outcomes among exposed workers (risks of hypertension and cardiovascular disease), based upon the interaction of lead exposures and genetic polymorphisms.
4. compare the differences in estimates of risk, on a population basis, between the gene:environment models and standard models of risk assessment for lead-induced hypertension.

Background. It is becoming recognized that variations in the genes encoding for important metabolizing enzymes may affect human response to toxicants, similarly to the well described interactions between these enzymes and individual response to pharmacotherapies. With the advent of greatly enhanced genetic information, as well as technologies to evaluate gene response (not solely gene structure, but also gene expression), we have the ability to utilize this information in risk assessment. However, at present we have relatively little information to determine the relevance of this information to risk assessment. That is, under what circumstances does incorporation of information on genetic polymorphisms actually affect a risk assessment (and by implication, influence risk based policy)? To answer this question, it is useful to conduct experiments to estimate the nature and magnitude of the impact of incorporating this information into specific risk assessments. To our knowledge, this has not been done in occupational health in a rigorous manner. We undertake this project from the perspective of enhancing worker protection through incorporation of genetic information, rejecting the alternative approach of worker segregation based on genotype.

Our hypothesis is that there is a some frequency of polymorphism, and some magnitude of phenotypic difference below which consideration of genetics is irrelevant for risk assessment

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purposes. We propose to utilize toxicological methods to investigate the extent to which a specific genetic polymorphism plays a significant role in affecting the toxicity of a specific occupational toxicant, and for which the population frequency of genetic polymorphisms is relatively great. Using physiological and molecular methods, we will determine the phenotypic significance of genetic polymorphisms in order to estimate the impact of genotype on response to toxicant exposure. This example may provide a means of calculating the significance, for risk assessment, of genetic variance that encodes measurable phenotypic difference.

Essential hypertension is a major cause of cardiovascular disease. Exposure to lead is among the environmental etiologies associated with hypertension. Epidemiological studies have demonstrated associations between elevated blood lead levels and blood pressure, as well as risks of hypertensive heart disease and stroke, among persons occupationally and environmentally exposed to lead (1, 2, 3, 4). Occupational lead exposures remain relatively high, in comparison to environmental exposures, and are within the range associated with increased risks of hypertension.

Physiologically, blood pressure homeostasis is regulated by the release of nitric oxide (NO, otherwise known as endothelial relaxing factor) from endothelial cells, resulting in vascular dilatation and decreased intravascular pressure (5). Inhibition of nitric oxide by endothelial nitric oxide synthase (eNOS) is associated with hypertension and increased vascular resistance (6, 7). The gene encoding eNOS is polymorphic, and some (but not all) studies have found associations between specific genetic polymorphisms and risks of heart disease (8, 9). The mechanisms by which lead induces hypertension are not fully known. There is evidence, from experimental studies, that lead can inhibit eNOS (10, 11).

Methods. We will utilize an example for which there is epidemiologic and genetic data, to test our hypotheses: the role of the Glu298Asp polymorphism in the eNOS gene in modulating lead-induced hypertension (8). Polymorphisms in the eNOS gene are associated, in some studies, with differential risks of hypertensive heart disease, presumably because of differences in the efficiency of nitric oxide production. However, the phenotypic consequences of genotypic differences in eNOS are not known, so that this will be part of this project..

1. **Effects of lead on eNOS and hypertension in an animal model.** We will investigate the role of eNOS in lead-induced hypertension first in guinea pigs, which are a frequently utilized model for the study of NO and cardiovascular function. In the first set of studies, cellular preparations of eNOS will be treated with lead in vitro, in concentrations ranging from 0.05 to 50 uM. The synthesis of NO from arginine will be quantitatively measured by sensitive radiochemical methods. In the second set of studies, the lowest effective concentration of lead (as an inhibitor of eNOS) will be tested in vitro for its effects on vascular tension, using preparations of guinea pig artery. In the third set of studies, adult guinea pigs will be exposed to lead in vivo, in exposures producing blood lead concentrations comparable to occupational exposures (25-50 ug/dL) and blood pressure will be monitored along with eNOS activity and NO levels.

2. **Effects of genetic polymorphisms on eNOS activity.** In these studies, we will pursue two approaches. First, we will collect whole blood spots, lymphocyte samples (immortalized for storage at –80 C), and plasma from anonymous donors. DNA will be extracted from blood spots and genotyped for the Glu298Asp polymorphism. Using material from persons homo- or

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heterozygous for this polymorphism, we will measure the following phenotypic indicators: circulating levels of NO in plasma of variant and wild type carriers; gene expression (enzyme levels) and enzyme activity of eNOS in the stored lymphocytes to determine the phenotypic consequences of genetic differences. Second, since the eNOS gene is sequenced and available through GenBank, we can make the mutant gene and insert it into a human or prokaryotic expression system, and then test the gene product in vitro for enzyme activity.

3. **Effects of genetic polymorphism on lead-induced inhibition of eNOS activity.** With a well-characterized gene product from the mutant form of the eNOS gene, we will test its sensitivity to lead. We will characterize the effects of genetic polymorphism by pharmacological analysis of IC_{50} and slope, in order to determine if the polymorphism has a significant effect on responsiveness to lead.

4. **Estimation of the impacts of genetic polymorphism on risks of lead induced hypertension.** Utilizing the information derived above, we will calculate the potential effects of the polymorphism on in vivo, human response to lead in terms of hypertension risk. This work will be done in collaboration with epidemiological colleagues knowledgeable in cardiovascular epidemiology and biostatistics. The next stage will be to consider the population implications of genetic polymorphisms by incorporating a term reflecting the estimated population frequency of genetic polymorphisms in eNOS in the US population, using published data on allelic frequencies in various ethnic groups. We will then compare the estimates of risk derived from incorporating genetic/phenotypic information with estimates developed by standard risk assessments of lead induced hypertension (3). This will permit us to test the overall hypothesis that incorporating information on the frequency and significance of specific genetic polymorphisms may influence risk assessment and risk management.

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